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# Selection of *Saccharomyces cerevisiae* strains for use as a microbial feed additive

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N. AGARWAL, D.N. KAMRA, L.C. CHAUDHARY, A. SAHOO AND N.N. PATHAK. 2000. *Saccharomyces cerevisiae* ITCCF 2094, NCIM 3052, 1031, 1032, NCDC 42, 45, 47, 49 and 50 were screened for their tolerance to pH 2·0–7·0, various concentrations (0·00, 0·10, 0·25 0·50 and 1·0%) of a mixture of acetic, propionic and butyric acids (70:20:10), and bile salts (0·00, 0·30, 0·60 and 0·90%). Low pH (2·0–4·0) and addition of organic acids or bile salts in the medium inhibited the growth of all the strains tested, but the percentage of inhibition was variable in the different strains of yeast. Two of the strains showing maximum tolerance, 42 and 49, were further tested for *in vitro* dry matter degradability (IVDMD) using green berseem, wheat straw and oat hay as substrates. *Saccharomyces cerevisiae* 49 enhanced the IVDMD of berseem and wheat straw whereas *S. cerevisiae* 42 was ineffective. Based on the results of the present experiment, *S. cerevisiae* NCDC 49 can be considered as the best strain which might tolerate the adverse conditions in the gastrointestinal tract when used as a live microbial feed supplement in the diet of the animals.

## INTRODUCTION

The effect of supplementation of the diet with microbial feed additives for the improvement of health and production of livestock has been studied for many years. Commonly used probiotics include *Saccharomyces cerevisiae* for enhancing the activity of beneficial microbes in the gastrointestinal tract, thus improving the digestibility of nutrients and production potential of the animals (Newbold *et al.* 1995; Singh *et al.* 1995; Wohlt *et al.* 1998), and *Lactobacillus* spp. for competitive exclusion of undesirable micro-organisms from the intestine, thus improving the health of the animal (Nader *et al.* 1993). There is a lot of variation in the performance of the same animal fed on different species of probiotic, or even the same species of probiotic but different strains. Newbold *et al.* (1995) observed that different strains of *S. cerevisiae* had different effects on rumen bacteria *in vitro* and in sheep. The probiotics entering the gastrointestinal tract have to face certain environmental constraints, and different strains of probiotic cultures differ in their sensitivity towards them. Some factors such as lysozyme, pancreatic enzymes, low pH, organic acids and bile salts, have been identified against which sensitivity of various cultures should be tested during selection for use as probiotics (Jin *et al.* 1998). The present study was designed to test various strains of *S. cerevisiae* for their tolerance to some of these factors and to study their effect on *in vitro* degradability of various feedstuffs.

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## MATERIALS AND METHODS

*Saccharomyces cerevisiae* ITCCF 2094 was obtained from the Indian Agriculture Research Institute, New Delhi, *S. cerevisiae* NCIM 3052, 1031 and 1032 were obtained from the National Chemical Laboratory, Pune and *S. cerevisiae* NCDC 42, 45, 47, 49 and 50 were obtained from the National Dairy Research Institute, Karnal. The yeast cultures were maintained on a medium containing: yeast extract, 3·5 g; peptone, 5 g; glucose, 10 g; agar, 20 g; distilled water, 1000 ml. Inocula for experimental work were prepared by growing the strains statically in broth medium (as above, without agar), incubated at 37 °C for 24 h.

### Tolerance to organic acids

The experiment to screen various strains of yeast for their tolerance to organic acids was conducted in two parts (a and b).

(a) Acetic, propionic and butyric acids were mixed in the ratio of 70:20:10, respectively, and the mixture added to broth medium at a concentration of 0·0,

0·10, 0·25, 0·50 and 1·00% (v/v). The broths containing the acid mixture at various concentrations were adjusted to pH 2·0, 3·0, 4·0, 5·0, 6·0 and 7·0 with 0·1 N HCl or NaOH. Tubes containing 7 ml broth were prepared in quadruplet for each treatment. Live yeast culture (1 ml) was mixed with 50 ml sterilized normal saline and 0·5 ml of this diluted culture was used for the inoculation of each tube. The tubes were incubated for 24 h at 39 °C and, after agitation on a vortex mixer, the absorbance at 540 nm was recorded to measure growth of the yeast cultures.

(b) The tubes for each treatment were prepared and inoculated in the same manner as in part (a) of the experiment. The tubes were incubated for 6 h at 39 °C, and another set of tubes containing normal medium without organic acids were inoculated with 0·5 ml of the 6 h treated culture. These freshly inoculated tubes were incubated for 18 h at 39 °C and the growth of the yeast cells was measured at 540 nm as described above.

#### Tolerance to bile salts

To study the tolerance of various strains of yeast to bile salts, the cultures were grown on solid medium containing bile salts at a concentration of 0·00, 0·30, 0·60 and 0·90%, respectively. The total viable count of the cultures was measured by the pour plate method using medium containing various levels of bile salts. The colonies were counted after 24 h incubation.

The percentage of inhibition was calculated as follows:

$$\% \text{ inhibition} = \frac{TVC_o - TVC_b}{TVC_o} \times 100$$

where $TVC_o$ = total viable count of culture grown on medium without bile salts, $TVC_b$ = total viable count of culture grown on medium with bile salts.

#### *In vitro* dry matter degradability

*In vitro* dry matter degradability (IVDMD) was estimated using the method of Goering and van Soest (1970). To study the effect of *S. cerevisiae* on IVDMD, 5 ml live yeast culture was added to the incubation mixture, which consisted of 40 ml McDougall's buffer (McDougall 1948), 10 ml rumen liquor and 0·5 g substrate. For controls, the live culture was boiled for 5 min to kill the cells and 5 ml of this dead yeast culture was added to the incubation mixture. The flasks were incubated at 39 °C for 24 and 48 h. The substrates tested were wheat straw, berseem (*Trifolium alexandrinum*) and oat hay (*Avena sativa*).

#### Statistical analysis

The data were subjected to analysis of variance (Snedecor and Cochran 1980) and significance differences were compared by Duncan's multiple test (Duncan 1955).

## RESULTS

In the presence of volatile fatty acids in the broth, the pH range which favoured growth was 4·0–7·0 for ITCCF 2094, NCIM 3052, 1031, 1032, NCDC 42, 45 and 50, and 3·0–7·0 for NCDC 47 and 49 (Table 1). The growth of NCIM 3052, 1031, 1032 and NCDC 50 was slow compared with other strains of yeast. The presence of the mixture of volatile fatty acids in the broth suppressed the growth of yeast in all cases. The suppression was enhanced as the concentration of fatty acids in the broth was increased from 0·1 to 1·0% acid mixture in the broth. When yeast cells were grown in the presence of the mixture of acids for 6 h and then inoculated into a broth without acids, some of the yeast cultures recovered and grew normally. Strains NCDC 42, 45, 47 and 49 were able to tolerate pH as low as 2·0 for 6 h (Table 1).

The presence of bile salts in the medium suppressed growth of all the strains, although complete inhibition (100%) was not observed even at the highest concentration of bile salts (0·9 g 100 ml$^{-1}$ solid medium). Among the strains tested, NCDC 49 showed least inhibition (only 9%) with 0·9 g bile salts 100 ml$^{-1}$ solid medium; the second most resistant was NCDC 42 (Table 2).

On the basis of the above tests, the two strains NCDC 42 and 49 were selected for determination of *in vitro* dry matter degradability of some common feeds. The fermenta-

**Table 1** Effect of pH and organic acids on the growth of *Saccharomyces cerevisiae*

| Strain no | Minimum pH at which growth occurred: Growth 24 h | 6 h shock |
|---|---|---|
| NCIM 3052 | 4·0 | – |
| NCIM 1031 | 4·0 | 5·0 |
| NCIM 1032 | 4·0 | Slow |
| ITCCF 2094 | 4·0 | 2·0 |
| NCDC 42 | 4·0 | 2·0 |
| NCDC 45 | 4·0 | 2·0 |
| NCDC 47 | 3·0 | 2·0 |
| NCDC 49 | 3·0 | 2·0 |
| NCDC 50 | 4·0 | Slow |

**Table 2** Percentage inhibition in the growth of probiotic cultures in the presence of bile salts

| | Bile salts (g 100 ml$^{-1}$) | |
|---|---|---|
| Strains of *S. cerevisae* | 0·60 | 0·90 |
| NCIM 1032 | 34·09 | 12·50 |
| ITCCF 2094 | 44·23 | 57·69 |
| NCDC 42 | 44·71 | 51·37 |
| NCDC 45 | 80·28 | 63·30 |
| NCDC 47 | 89·37 | 87·77 |
| NCDC 49 | 25·00 | 9·09 |
| NCDC 50 | 33·77 | 52·98 |

tion was very fast in the case of berseem and oat hay compared with wheat straw after incubation for 24 h. Between 24 and 48 h, the rate of fermentation of berseem and oat was very slow, whereas fermentation of wheat straw continued steadily, thus narrowing the differences in IVDMD of the three substrates. The inclusion of NCDC 49 significantly ($P < 0{\cdot}01$) increased IVDMD of wheat straw and berseem, whereas oat hay degradability remained unaffected at 24 h of incubation. At 48 h of incubation there was no difference in IVDMD of the three substrates tested, with and without yeast (Table 3). As yeast did not show any effect at 48 h, incubation was done only for 24 h with NCDC 42. The degradability of the three substrates was not affected by inclusion of NCDC 42 in the incubation mixture.

## DISCUSSION

Amongst the factors identified for the screening of probiotic cultures for their use as feed supplements, resistance to low pH, organic acids and bile salts were considered to be of prime importance for the selection of strains (Jin *et al.* 1998). According to Newbold *et al.* (1990) and El Hassan *et al.* (1993), yeast cannot multiply in the rumen. Aramble and Tung (1987) suggested that the temperature (39 °C) and the chemical composition of the rumen liquor may be responsible for preventing the yeast from multiplying. However, the positive effect of yeast supplementation on cellulose-degrading enzymes and feed degradability (Maurya 1993) indicates that cells of *S. cerevisiae* remain active in the rumen and have a stimulatory effect upon cellulose-degrading bacteria. Several strains of yeast have been screened for the parameters which they might encounter while passing through the gastrointestinal tract of the animals when used as a feed aaditive.

The presence of volatile fatty acids in the medium adversely affected the growth of yeast cells even at the lowest concentration. The adverse effect was greater at low pH. This might be due to the fact that at a pH lower than the $pK_a$ value of the organic acids, a major portion of the acid is in the non-ionized form, which is permeable through the cell membrane of microbes. Once non-ionized acid is inside the cell where the pH is near neutral, acid ionizes and hence, cannot come out of the cells (Levine and Fellers 1940; Warth 1977). Therefore, at lower pH there is a constant influx of organic acids into the cells which ultimately results in death. Among the strains of *S. cerevisiae* tested in the present study, four strains were

**Table 3** Effect of live yeast culture (*Saccharomyces cerevisiae*) on the *in vitro* dry matter degradability of various feedstuffs

| Substrate | Incubation time (h) | Control | Treated | Significance |
|---|---|---|---|---|
| *S. Cerevisiae* NCDC 49 | | | | |
| Wheat straw | 24 | 17·06 ± 1·72$^a$ | 25·14 ± 1·13$^a$ | ** |
| | 48 | 36·17 ± 1·46$^b$ | 38·31 ± 0·46$^b$ | NS |
| Oat | 24 | 30·01 ± 4·10$^a$ | 32·59 ± 1·97$^a$ | NS |
| | 48 | 45·30 ± 2·55$^b$ | 32·59 ± 1·97$^b$ | NS |
| Berseem | 24 | 34·58 ± 2·09 | 45·03 ± 1·44 | ** |
| | 48 | 40·57 ± 2·13 | 41·70 ± 1·95 | NS |
| *S. Cerevisiae* NCDC 42 | | | | |
| Wheat straw | 24 | 15·81 ± 1·47 | 15·65 ± 1·66 | NS |
| Oat | 24 | 41·77 ± 1·13 | 38·61 ± 0·53 | NS |
| Berseem | 24 | 31·82 ± 0·53 | 33·13 ± 0·73 | NS |

Means bearing different superscript in a column differ significantly ($P < 0{\cdot}01$).
$^*P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, NS, non significant.

found to tolerate a shock of pH 2·0 for 6 h, and two strains could initiate growth at pH 3·0.

The highest concentration of bile salts used for the screening of yeast cultures was 0·9%, which is much higher than that found in the intestines of most domestic animals. Gilliland *et al.* (1984) considered 0·3% bile salts as a critical concentration for the screening of resistant strains. All the strains of *S. cerevisiae* showed some growth, even at the highest concentration of 0·9% bile salts, but the level of resistance was variable, being maximal in NCDC 49.

The two strains NCDC 42 and 49, selected on the basis of screening, behaved differently when tested for *in vitro* dry matter degradability; an increase in dry matter degradability of wheat straw and berseem was shown with NCDC 49, whereas NCDC 42 was ineffective. Such a behavioural anomaly among strains of the same species of yeast showing little difference in the level of resistance to low pH, volatile fatty acids and bile salts, indicates that this should also be considered as an important parameter for the screening of probiotic cultures.

Based on the above experiments, *S. cerevisiae* NCDC 49 can be considered as the best among the different strains tested which might be able to tolerate the adverse conditions of the gastrointestinal tract of animals and serve as a good agent for use as a microbial feed supplement.

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